STUDIES ON EXOSMOSIS

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In experiments on permeability where the turgidity of plant cells or their osmotic pressure (as judged by that of a solution just concentrated enough to plasmolyze them) is used as a criterion, there is an important source of error, which is usually overlooked. This lies in the possibility that osmotically active substances may diffuse out of the cell.

It was noted as early as 1860 by Knop, in connection with water cultures, that plant organs bathed by distilled water may give off substances to it. This phenomenon, usually termed exosmosis, may take place when tissues which are not normally in contact with water are placed in contact with distilled water or dilute solutions. Thus Wächter (7), found that strips of onion bulb scale gave off sugars to distilled water, and that this exosmosis was hindered by 0.1 to 0.4 *M* solutions of potassium and sodium chlorides and potassium nitrate.

Recently True and Bartlett (4, 5, 6) have made a thorough investigation of the intake and outgo of salts from roots of field peas grown in distilled and river water, and in solutions (mostly 0.001 *M*) of various salts singly and in combinations. A somewhat similar investigation by Merrill (1, 2) included the study of the effects of salt concentrations as high as 0.1 *M*.[1]

In all these experiments solutions were used whose concentration was far below that necessary to cause plasmolysis, and the effects were observed after periods up to fifty days, at which time the pure salt effects might be obscured by the readjustments of the organism to its change in environment. They do not, therefore, enable us to distinguish the immediate effects of salts on the plasma membrane, nor do they help us to determine the possible rôle of exosmosis in experiments whose duration is a matter of a few hours at most, as is the case in most experiments in which the turgidity of cells or their recovery from plasmolysis is used as a criterion of their permeability.

[1] It is not known to what extent the results (in all the cases here cited) may be due to the death of superficial cells of the roots.

A great many conclusions as to the nature of protoplasm or its surface layer (the so-called "plasma membrane") have been drawn from experiments on plasmolysis. But since the alterations in turgidity or in degree of plasmolysis may not only be increased by the entry ("endosmosis") of osmotically active substances from the solution bathing the cell, but may also be decreased by outward diffusion of similar substances ("exosmosis"), the rate of which may be altered by the plasmolyzing agent, it seemed highly desirable to observe the effect on exosmosis immediately following the application of solutions isotonic with the cells of the material used.

A series of such experiments was conducted in which the exosmosis of electrolytes into distilled water from strips of peduncles of the dandelion (*Taraxacum officinale* Weber)[2] was determined immediately following a previous treatment with distilled water or with sodium, calcium, or cerium chlorides.

Method and Precautions

In these experiments the best grade of water distilled from glass was used; the salts were Baker's "analyzed" sodium chloride, Kahlbaum's calcium chloride, and Merck's "Reagent," cerium chloride. The solutions were made up with a maximum error of 0.5 percent. That this accuracy was sufficient will be seen from the fact that a change in the concentration of the $CaCl_2$ solution from 3 percent below to 3 percent above that isotonic with the sodium chloride solution used produced no appreciable difference in the results of the experiment. Solutions were considered to be isotonic with the cells when there was for a few seconds a barely perceptible decrease in the curvature, as observed by the use of a microscope, of freshly cut strips of peduncle on immersion in the solution. A detailed discussion of the method of determination, and of the accuracy and significance of this criterion will be presented in a subsequent paper.

A peduncle was cut into pieces 5 cm. in length, and these were cut longitudinally into as many strips as there were solutions to be investigated; one strip from each piece was placed in each solution. About fifteen or twenty pieces of peduncle were so used. Since relative results only were sought, the number of strips used was not important; it was only necessary to divide each piece accurately, so

[2] The dandelions were grown in the greenhouse from wild plants dug up in the autumn. The plants were in the height of flowering when the material was used.

that the aggregate amount of material should be the same in the different solutions. The strips were protected from evaporation until all were cut; each lot was then placed in a test-tube, rinsed with distilled water which was allowed to drain off for thirty seconds, and the solutions were then poured in.

In these experiments three solutions were used: sodium chloride 0.22 *M*, calcium chloride 0.16 or 0.17 *M*, cerium chloride 0.050 *M*. In the control experiment distilled water took the place of a salt solution. After a period of from fifteen to twenty-five minutes these solutions were poured off and the material was rinsed three times with distilled water, the second change remaining in contact with the tissue two minutes. The last rinsing was allowed to drain off for thirty seconds, and then 13.0 cc. of distilled water placed in each test-tube. This amount was just sufficient to cover the strips of tissue, which were packed loosely in the bottom of the test tube. At the end of fifteen minutes the distilled water was poured off into a specially constructed U-tube designed to contain 13 cc. of solution, and its conductance determined. The solution was then returned to the material, and its conductance determined in a similar manner at suitable intervals.

Preliminary experiments indicated the possibility that some substance gathered on the electrodes, forming there a highly resistant layer. It was therefore decided to interpose parchment thimbles between the electrodes and the solution. These thimbles were kept in distilled water, and were placed (filled with distilled water) in the expanded ends of the U-tube just prior to each measurement. Figure 1 will show the arrangement of the U-tube, the bright platinum electrodes, and the solutions. It will be seen that the current traversed always the same amount of distilled water and the same length of column of solution. The distilled water in the parchment thimbles was not changed during a single set of three or four readings; it was possible at the end of a set to duplicate so closely the first reading taken, that it was evident that the diffusion of electrolytes into the distilled water in the thimbles introduced no appreciable error.

The largest source of error was that introduced by a certain amount of distilled water which it was impracticable to remove from the outside of the parchment thimbles before introducing them into the U-tube. The error thus introduced was not, however, sufficient to cause any significant variation in the results of the experiment. The

U-tube was placed in a constantly stirred water bath, whose temperature was determined to within 0.1° C., and the conductance determined by means of the ordinary arrangement of a slide-wire bridge, Nernst string inductorium, standard 1,000-ohm coil and telephone receiver. The error was less than 1 percent. The fluctuations in temperature were not great enough to justify the introduction of a temperature correction.

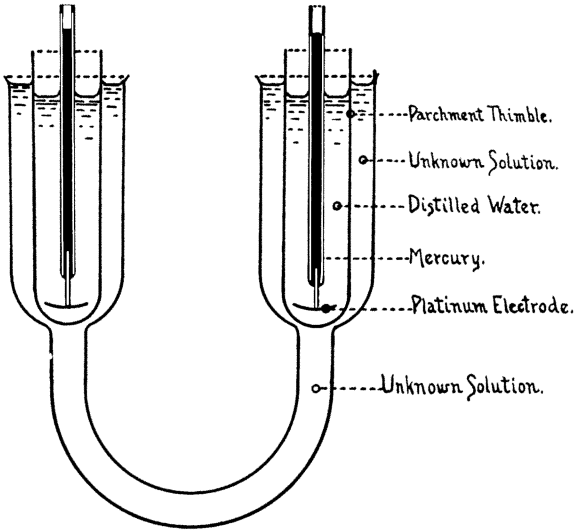

FIG. 1.

In the later experiments it was found that the parchment thimbles could be omitted, with a corresponding gain in the accuracy of the readings. The conductance was then determined between the two electrodes, now immersed directly in the solution. This fact prevents the direct comparison of readings taken by the different methods, but, as the comparative values remain unchanged, does not destroy the significance of the experiments.

It is obvious that the total increase in the conductance of the distilled water in contact with material which has been immersed in a salt solution will measure not only exosmosis from the protoplasm, but also diffusion from the intercellular material (*i. e.*, all non-protoplasmic material, including cell walls and intercellular spaces). This diffusion from intercellular material will change the conductance

of the distilled water to a degree dependent on the concentration and molecular conductivity of the salt solution used, and will be absent in the control experiment, in which distilled water takes the place of a salt solution.

It is possible, however, to determine the duration of this diffusion, and by comparing the rate of change of conductance subsequent to its practical completion to gain an insight into the effect of salts on exosmosis from the protoplasm. Tissue which has been treated with

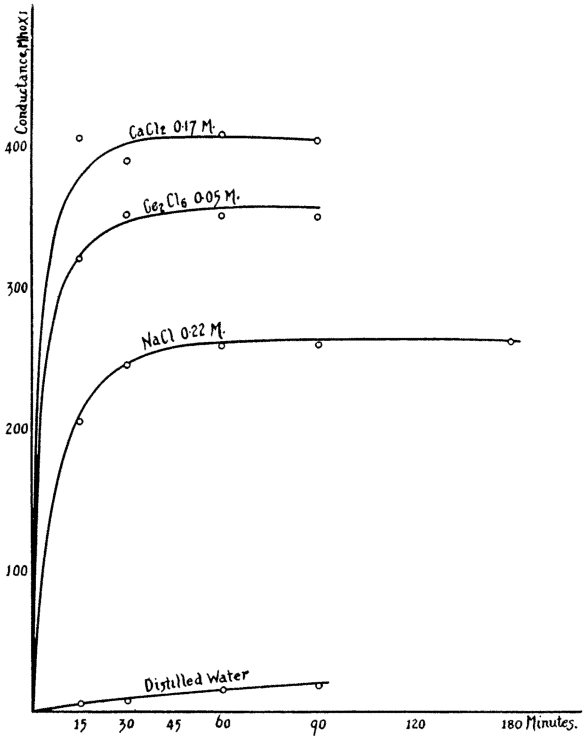

FIG. 2.

distilled water for twenty-four hours has practically ceased to give off electrolytes to the distilled water. If such tissue be treated with salt solutions, as described above, it will be found that after thirty minutes the rate of change of conductance parallels that found for the same material treated with distilled water. This fact is apparent from the data in Table 1, and is represented graphically in Figure 2,

in which the ordinates represent conductance (in ohms) and the abscissæ the time which elapsed between placing the material in distilled water and the determination of the conductance.

TABLE I.

Diffusion of Salts from Tissue Leached in Distilled Water for a Period of Twenty-three Hours Previous to Treatment with the Salt Solutions

Time in Minutes After Transfer to Distilled Water	Conductance in Ohms $\times 10^7$ of Distilled Water in Contact with Tissues Previously Exposed 25 Min. to:			
	NaCl 0.22*M*	$CaCl_2$ 0.17*M*	Ce_2Cl_6 0.05*M*	Distilled Water
15	205	405	320	5
30	245	389	351	7
60	258	407	350	15
90	259	403	350	18
180	261	—	—	—

In this experiment the differences of conductance are caused almost exclusively by the diffusion from the tissues of the salts with solutions of which they have been treated; the conductances are therefore closely proportional to the conductances of these solutions. The experiment shows that diffusion of the absorbed salt from the tissues is completed within thirty minutes, and the increase of conductance in the different solutions subsequent to this time may be attributed largely to exosmosis from the protoplasm.

RESULTS

If strips of dandelion peduncle be immersed (after momentary rinsing) in distilled water there is a steady exosmosis of electrolytes, and the rate of this exosmosis decreases gradually and without sudden change during the duration of the experiment, a matter of from six to eight hours. Since the protoplasm is normally in equilibrium with a more or less concentrated solution permeating the intercellular substance, the replacement of this solution by distilled water will necessarily lead to a disturbance of the equilibrium, and may, without causing any marked change in the normal permeability of the protoplasm, lead to an abnormal diffusion of substances from the cell, or, in other words, to an abnormal exosmosis. A previous treatment of the tissue with an isotonic solution of sodium chloride has the effect of accelerating this exosmosis, which, on the other hand, is inhibited by an isotonic solution of calcium chloride.

The first effect of cerium chloride is, like that of calcium, an inhibition of the exosmosis, but this effect is quickly reversed, and the rate of exosmosis becomes greater than that from any other of the lots of material. This is probably due to the not inconsiderable toxicity of the solution of cerium chloride, enough of which probably remained in the protoplasm to cause injury, with consequent greatly increased exosmosis. The effect of sodium chloride is only temporary, disappearing within one and one quarter hours after the removal of the tissue from the sodium chloride solution.

TABLE 2.

Effect of Salts on Exosmosis from Unleached Tissue

Time in Hours After Transfer to Distilled Water	Conductance in Ohms, $\times 10^7$, of Distilled Water in Contact with Tissues Previously Exposed 20 Min. to :			
	NaCl 0.22 *M*	$CaCl_2$ 0.17 *M*	Ce_2Cl_6 0.05 *M*	Distilled Water
0.50	28.0	44.5	24.0	8.0
1.25	41.0	49.5	27.0	16.0
3.00	48.0	51.5	40.0	26.0
4.57	51.0	53.0	45.5	31.0
5.90	51.5	53.3	47.0	32.0

The data are given in Table 2, and are graphically presented in Figure 3, in which the ordinates represent the total gain in conductance at intervals of time after the end of the period of thirty minutes which was allowed for diffusion of salts from the intercellular material.

A consideration of the fact that after the first thirty minutes the exosmosis from tissue which had been treated with calcium chloride was less than that from tissue which had not been in contact with any salt solution, shows that the substance causing the increase of conductance was not, or at least only to an extremely small extent, the salt used; the data therefor show that sodium salts increase, and calcium salts decrease the permeability of the protoplasm to substances other than themselves.

By analogy with the experiments of Osterhout (3) on *Laminaria* it should be possible to find some mixture of salts which, in a solution of the proper concentration, would leave the permeability of the dandelion protoplasm unaltered. Such a combination was found. It was not possible to determine exactly its optimum constitution; but a solution consisting of 80 parts sea water and 20 parts of a 0.52 *M* solution of calcium chloride, diluted to 21/52 of its original concen-

tration, was found to present approximately the desired "normal" conditions for the protoplasm of the dandelion. In Table 3 will be found the constitution of this solution in gram molecules per liter, and

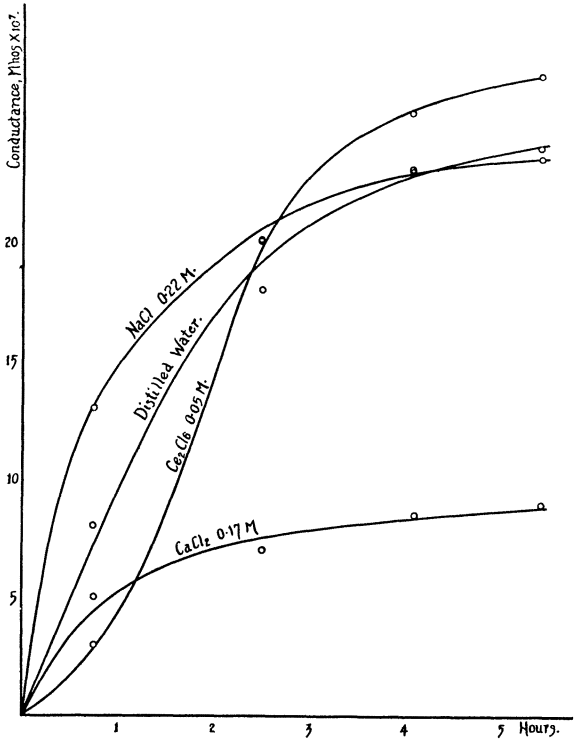

FIG. 3.

TABLE 3

Constitution of a Solution Favorable to the Protoplasm of Taraxacum officinale, and That of Sea Water at the Same Dilution

Salt	Sea Water		Solution	
	Gm. Mols per Liter	Percent of Total Gm. Mols	Gm. Mols per Liter	Percent of Total Gm. Mols
NaCl	0.2020	86.2	0.1608	68.4
$CaCl_2$	0.0046	2.0	0.0465	19.8
$MgCl_2$	0.0158	6.7	0.0158	6.7
$MgSo_4$	0.0077	3.3	0.0077	3.3
KCl	0.0046	1.9	0.0046	1.9
Tota	0.2347	100.1	0.2354	100.1

the percentage of the total number of gram molecules present in the form of each of the constituent salts. The corresponding figures for sea water diluted to the same extent are given for comparison.

The effect of this solution on exosmosis from the protoplasm (after the first thirty minutes) does not differ appreciably from that of

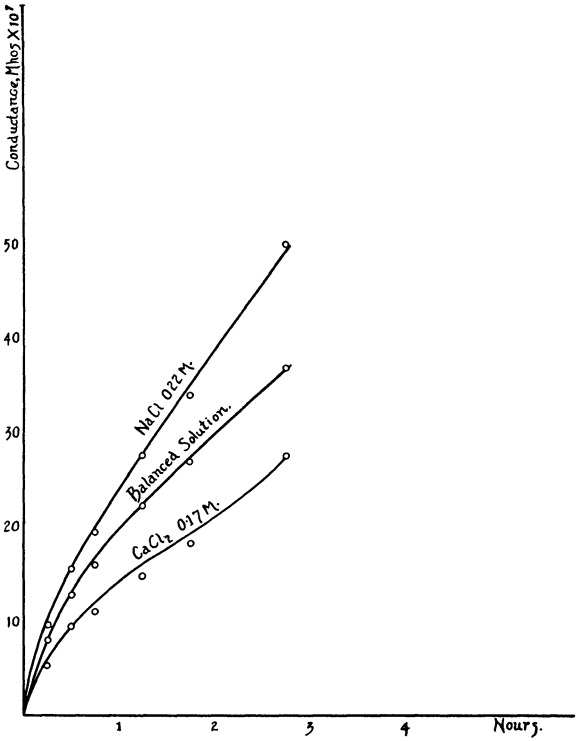

FIG. 4.

distilled water. The rate of exosmosis lies always intermediate between those for sodium and calcium chlorides, as will be seen from the data given in Table 4, and graphically presented in Figure 4.

Results wholly analogous with those given above were secured after much shorter exposures to the salt solutions. Effects could be detected even after a four-minute exposure to a sodium chloride solution.

TABLE 4

Effect of Balanced and Pure Salt Solutions on Exosmosis from Unleached Tissue

Time in Hours After Transfer to Distilled Water	Conductance in Ohms, $\times 10^7$, of Distilled Water in Contact with Tissues Previously Exposed 30 Min. to:		
	Balanced Solution	NaCl 0.22*M*	$CaCl_2$ 0.17*M*
0.75	55.0	78.5	73.5
1.00	63.0	88.0	78.7
1.25	67.8	94.0	83.0
1.50	71.0	98.0	84.5
2.00	77.8	106.0	88.3
2.50	81.8	112.3	91.7
3.50	91.7	128.4	100.1

SUMMARY

1. Sodium salts increase the rate of exosmosis of other electrolytes from the protoplasm of *Taraxacum officinale.*

2. Calcium salts decrease this rate.

3. A solution may be prepared consisting of a mixture of various salts in proportions such that, when used at a concentration isotonic with the protoplasm, it causes no appreciable alteration in the permeability of the plasma membrane of *Taraxacum officinale.*

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